PAN-PACIFIC ENTOMOLOGIST
74(3): 150–156, (1998)

AN EXAMINATION OF THE PHYLOGENETIC UTILITY OF TAXONOMIC TRAITS IN MELANOPLINE GRASSHOPPERS (ORTHOPTERA: ACRIDIDAE)

W. CHAPCO AND S. J. GUENTHER
Department of Biology, University of Regina,
Regina, Saskatchewan, S4S 0A2 Canada

Abstract—A cladistic analysis of several morphological traits commonly used in the identification of melanopline grasshoppers was performed in order to investigate their phylogenetic utility and to provide a basis for the eventual comparison with DNA-derived phylogenies.

Key Words—Insecta, Orthoptera, *Melanoplus,* grasshoppers, cladistics.

" . . . at present the exact relationship of the many species is unknown"

A. R. Brooks 1958

Brooks's statement regarding evolutionary relationships among members of the genus *Melanoplus* Stål is still true today. The most striking feature of the subfamily Melanoplinae, to which the genus belongs, is the vast array of shapes and sizes of male genitallic parts (Brooks 1958). Melanoplines also differ in several body characters (Vickery & Kevan 1986), egg and egg pod characteristics (Onsager & Mulkern 1963), nymphal traits (Handford 1946), and isozyme patterns (Chapco 1989). A few taxonomic keys exist (e.g., Brooks 1958, Vickery & Kevan 1986, Helfer 1987) which incorporate subsets of morphological features (particularly external male terminal structures), but to our knowledge, these traits have yet to be analyzed from an evolutionary perspective. Since a goal of this laboratory is eventually to construct a DNA-based evolutionary tree of melanoplines, an analysis of conventional traits would be extremely useful for comparative purposes. To this end, the results of a cladistic analysis of a small data set are presented and the phylogenetic utility of characters used in taxonomic identification examined.

MATERIALS AND METHODS

This study focuses on 11 species of melanopline grasshoppers, chosen on the basis of the availability of local living specimens and taxonomic data in the literature. Species are: *Melanoplus angustipennis* (Dodge), *M. bivitattus* (Say), *M. confusus* Scudder, *M. dawsoni* (Scudder), *M. femurrubrum* (De Geer), *M. gladstoni* Scudder, *M. infantilis* Scudder, *M. packardii* Scudder, *M. sanguinipes* (Fabricius), *Phoetaliotes nebrascensis* (Thomas), and *Hesperotettix viridis* Scudder. Ten characters frequently used in identification and representing a diversity of biological features were selected (Table 1). The stability of the derived relationships was examined by including additional traits, morphometric and electrophoretic, to form a larger data set. Traits 11–20 are based on Guenther's (1994) analysis of several morphometric characters. Femur length is used as a surrogate for "body size" (Harrison 1986), a legitimate taxonomic character (Simon 1983; Maurer et al. 1992). Traits 13–20 are ratios of linear measurements and reflect

Table 1. Characters and character states for melanopline grasshoppers.

Character	States
1. Dorsal angle of ovipositor[a]	0 (<114°) 1 (114°–136°) 2 (>136°)
2. Micropyle of egg[b]	0 (not conspicuous), 1 (somewhat conspicuous), 2 (conspicuous)
3. No. columns of eggs/pod[b]	0 (2) 1 (2 or 3) 2 (4)
4. No. eggs/pod[b]	0 (<16) 1 (16–30) 2 (>30)
5. Markings on outer face of hind femora of late instars[c]	0 (spotted) 1 (broken) 2 (solid)
6. Cerci type (males)[a]	0 (plain), 1 (elaborate)
7. Lateral surface of cerci (males)[d]	0 (not spatulate) 1 (mildly to strongly spatulate)
8. Furculae length[d]	0 (short) 1 (medium) 2 (long)
9. Antennal crescent[d]	0 (divided) 1 (complete)
10. Notch of eighth sternite (females)[d]	0 (very shallow to shallow) 1 (moderate to deep)
11. Body size (hind femoral L)[d]	six homogeneous sets (coded 0–5)
12. No. of tibial spines[d]	five homogeneous sets (coded 0–4)
13. Hind femoral L/W[d]	seven homogeneous sets (coded 0–6)
14. Hind femoral L/hind tibial L[d]	five homogeneous sets (coded 0–4)
15. Pronotal max W/min W[d]	three homogeneous sets (coded 0–2)
16. Prozonal L/metazonal L[d]	seven homogeneous sets (coded 0–6)
17. Interocular D/epistomal suture L[d]	three homogeneous sets (coded 0–2)
18. Subocular fissure L/head W[d]	four homogeneous sets (coded 0–3)
19. Dorsal eye D/interocular D[d]	four homogeneous sets (coded 0–3)
20. Head W/pronotal L[d]	seven homogeneous sets (coded 0–6)
21. Combinations of Ldh[e] alleles	seven states (coded 0–6)
22. Combinations of alpha-GPdh[f] alleles	three states (coded 0–2)

D, L, W = distance, length and width, respectively. Sources: a—Brooks (1958); b—Onsager & Mulkern (1963); c—Handford (1946); d—Guenther (1994); e—Chapco (1984); f—Chapco et al. (1987).

shape characteristics of different body parts. Guenther (1994) showed that, unlike linear characters, ratios vary little between sexes and geographic location and are, therefore, expected to be more reliable. In any case, measurements in this study were restricted to those of female insects collected within one large area in southern Saskatchewan. Traits 21 and 22 are electrophoretic characters for which inheritance patterns have been previously established (Chapco 1984, Chapco et al. 1987).

For cladistic purposes, continuous traits were coded employing Simon's (1983) homogeneous subset coding scheme. This involved assigning an integral value to a taxon according to its inclusion in a homogeneous subset. Subsets were established by *a posteriori* multiple comparisons tests which, in the majority of cases, were parametric. In a few situations (< 6%), continuous traits were not normally distributed necessitating the use of non-parametric methods. Electrophoretic loci were treated as separate characters with allelic combinations defining different character states (Buth 1984). Cladistic analyses using the programs. MacClade (Maddison & Maddison 1992) and PAUP (Swofford 1993), were applied to the two overlapping sets of data. The "BRANCH AND BOUND" search command of PAUP was employed to find the tree or set of trees, explained by the least number of character state changes. Since there was no *a priori* reason for considering any one species as an outgroup, a mid-rooting scheme available in PAUP was adopted. Hence, no character state was regarded as ancestral or derived. The reliability of putative associations was ascertained by the use of "decay indices" (Donoghue et al. 1992). A decay index is the number of steps additional to the number in the shortest tree(s) required to collapse a tree branch node. All characters, except LDH (for which no transformation pattern was evident), were treated as ordered. To see what effect this might have, an analysis treating all characters as unordered was also performed.

Results and Discussion

Cladistic analysis of the 11 species × 10 character data set (Table 2) yielded two equally parsimonious trees, each of length 29 and consistency index 0.55. A majority-rule consensus tree is presented in Figure 1.1. Mid-point rooting identified two main clusters A and B, the former consisting of the 7 species: *M. angustipennis* to *H. viridis,* and the latter consisting of the 4 species: *M. bivitattus* to *M. femurrubrum.* Within A, pairs (*M. angustipennis, M. packardii*), and (*M. confusus, M. infantilis*) emerge, and are strongly supported by decay indices of 6^+ and 6 steps, respectively. However, relationships among those pairs and remaining taxa in A are unresolved. Within B, the branching pattern (((*M. bivitattus, P. nebrascensis*), *M. dawsoni*), *M. femurrubrum*) is indicated, but this structure breaks down when all trees 2 steps greater than the most parsimonious trees are considered. The distinction between clades A and B disappears in trees that are 4 steps longer than the shortest trees.

Relationships are modified if the 10 traits are treated as unordered (Fig. 1.2). Parsimony analysis resulted in 17 equally parsimonious trees, each of length = 27 and consistency index = 0.60. Clades (*M. angustipennis, M. packardii*), and (*M. confusus, M. infantilis*) are still retained, supported in each case by decay indices > 3. The major difference from Figure 1.1 is the relocation of *H. viridis* from group A to group B.

Table 2. Character state data for 11 melanopline species. The 22 characters and their state codes are described in Table 1; missing values are indicated by ?s.

	Character number																					
Species[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
ang	0	0	0	1	0	0	1	1	0	1	?	?	?	?	?	?	?	?	?	?	3	2
biv	2	2	2	2	2	1	0	0	0	1	5	2	4	1	0	2	3	3	5	2	4	1
conf	0	1	0	0	1	1	1	1	1	1	3	1	3	3	1	2	5	4	3	5	5	1
daws	2	2	0	0	2	0	0	1	0	0	0	3	3	3	4	4	4	2	2	6	2	1
fem	1	2	0	1	2	0	0	2	1	0	2	3	3	3	3	0	4	2	1	4	1	2
glad	1	0	0	1	1	0	0	0	0	1	1	0	0	3	0	1	2	3	2	6	6	2
inf	1	1	0	0	1	1	0	0	1	1	0	0	1	3	5	3	1	1	1	8	7	0
pac	0	0	1	1	0	0	1	1	0	1	5	0	3	3	0	2	4	4	3	1	3	2
sang	1	0	0	1	1	0	0	1	0	0	4	2	2	3	2	0	4	3	1	3	1	1
Pn	2	2	0	1	2	0	0	0	0	0	2	2	4	2	6	5	0	0	4	7	?	?
Hv	2	0	0	1	0	0	0	0	1	0	4	0	4	0	0	3	6	4	0	0	?	?

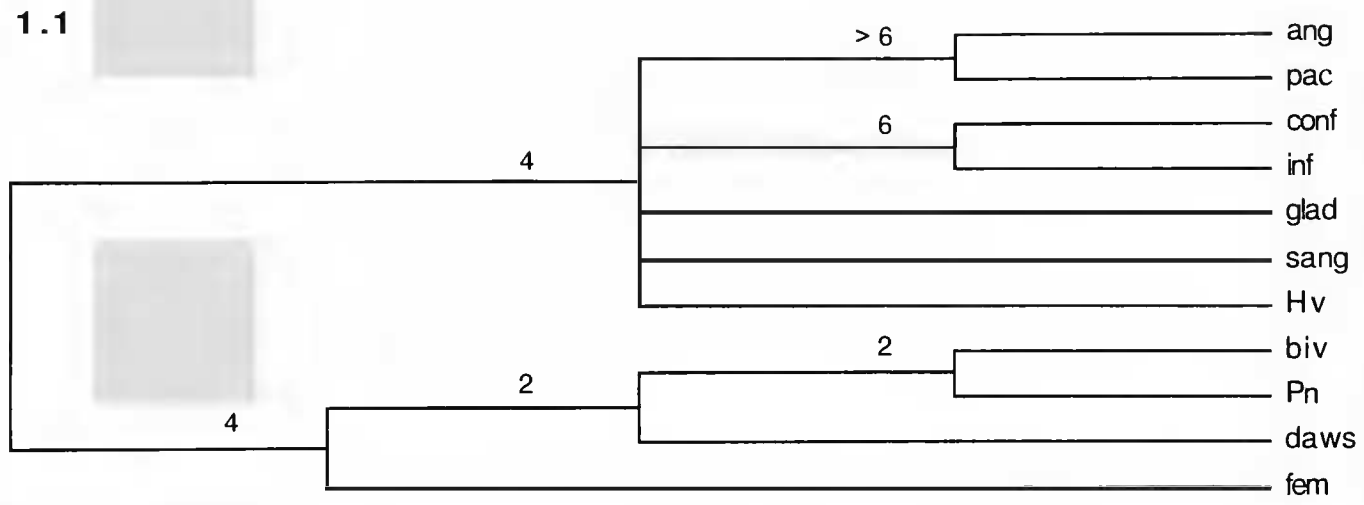
(a) = listed as follows: ang = *Melanoplus angustipennis,* biv = *M. bivitattus,* conf = *M. confusus,* daws = *M. dawsoni,* fem = *M. femurrubrum,* glad = *M. gladstoni* Scudder, inf = *M. infantilis* Scudder, pac = *M. packardii* Scudder, sang = *M. sanguinipes,* Pn = *Phoetaliotes nebrascensis,* Hv = *Hesperotettix viridis.*

In the analysis of the expanded data set (Table 2), a single most parsimonious tree of length 136 and consistency index 0.54 was obtained (Fig. 2.1). A few comparisons with Figure 1.1 are worth noting. Support for the (*M. angustipennis, M. packardii*) clade remains, which is not surprising since the expanded data set for *M. angustipennis* is largely incomplete, although both species have identical isozyme patterns. The association between *M. confusus* and *M. infantilis* has disappeared, but now there is a strongly supported relationship between *M. bivitattus* and *H. viridis.* The net result is that *M. bivitattus* and *M. infantilis* have reversed their affiliations with A and B. The distinction between these major groups, however, is supported by a decay index of only one.

Treating all traits as unordered, 11 trees each of length 94 and consistency index 0.78 were identified: a consensus tree is presented in Figure 2.2. It would appear that there is considerable realignment of taxa with respect to Figure 2.1.

It is tempting, therefore, to conclude that little confidence can be placed in any of the trees. Nevertheless, certain general features are evident if all four figures are compared simultaneously. It can be seen that *M. angustipennis, M. packardii,* and *M. confusus* are always separated from *M. dawsoni, M. femurrubrum,* and *P. nebrascensis,* although relationships among members within each triad depend on the data set and how characters are treated. Affiliations of the remaining taxa with either group are inconsistent. A surprising consistent result is the intermingling of the two non-*Melanoplus* species within each cluster. If true, the result might put into question the monophyly of the major genus. It is also possible that the internal placements of these two species are artifacts of mid-point rooting and that the true root is along the branch leading to either species.

Most characters exhibit multiple character state changes (not shown) in different branches of the trees. What this widespread homoplasy means is that none of the



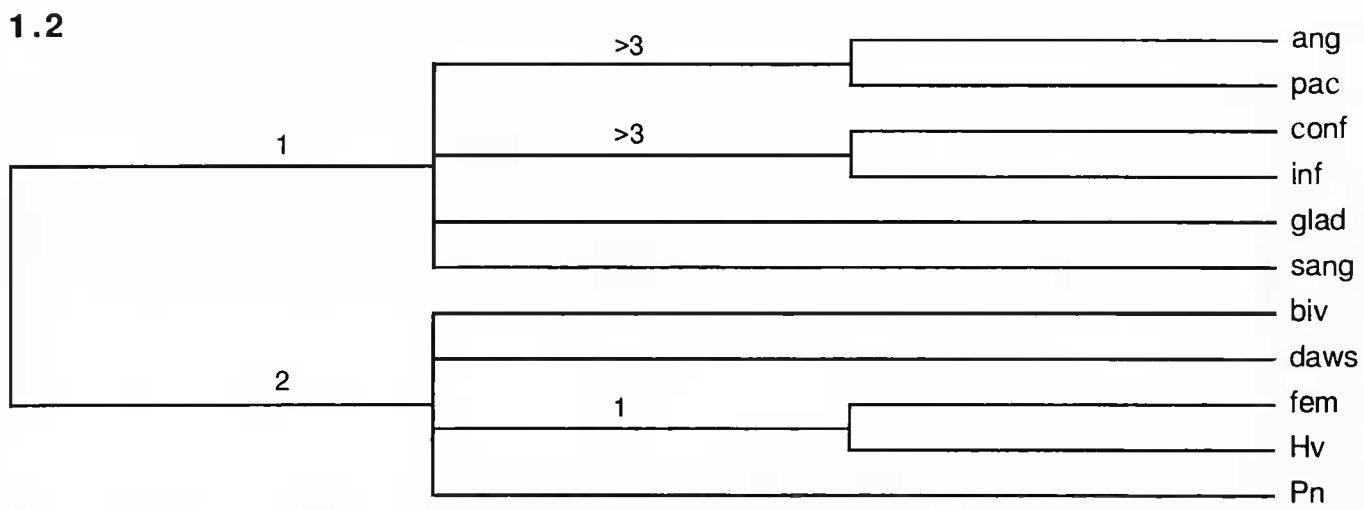
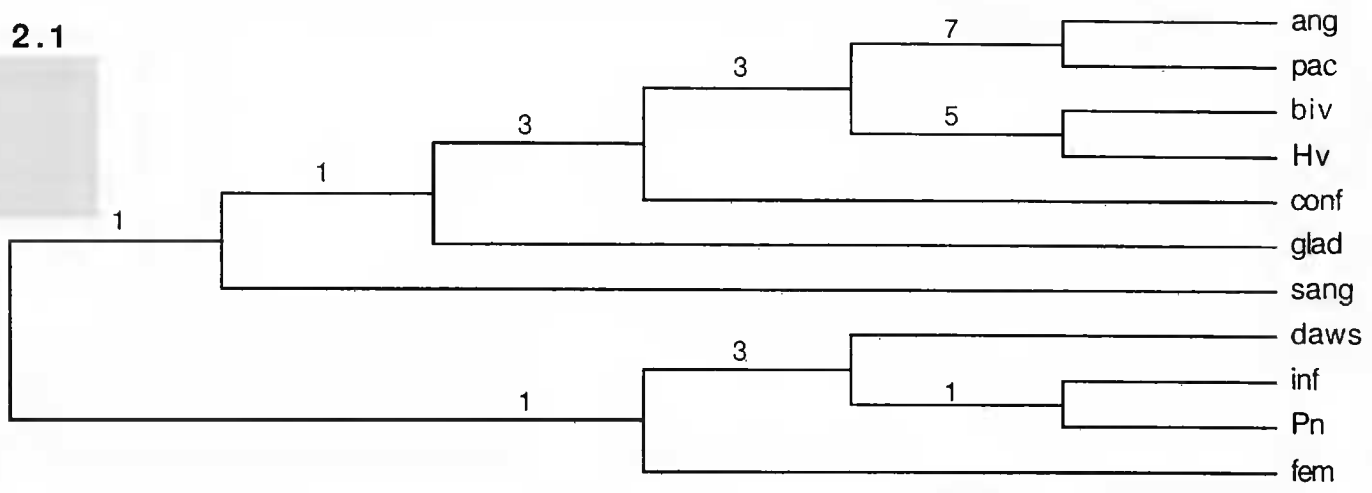


Figure 1.1. Majority rule consensus tree of two equally parsimonious trees, each of length 29 and consistency index of 0.55, depicting relationships among 11 melanopline grasshopper species. Trees are based on 10 (mostly) ordered characters used in taxonomic identification.

Figure 1.2. (Same data set with characters treated as unordered) Majority rule consensus tree of 17 equally parsimonious trees, each of length 27 and consistency index 0.60. Numbers on branches in both figures refer to decay indices. Taxon abbreviations are defined in Table 2.

clusters can be defined by a unique collection of shared character states. Does this imply, therefore, that the characters chosen for analysis are phylogenetically inutile? While the goal of traditional Hennigian cladistics is to erect monophyletic group on the basis of a set of derived traits that are mutually consistent, this is not necessarily the focus of modern cladistics (Quicke 1993). With respect to our data, one to several parsimonious trees, depending on the character set and orderedness of the characters, emerged. Some branches were robustly supported and some weakly so. At the very least, these trees and assumptions underlying the traits can serve as hypotheses which will be tested when DNA-based trees have been obtained. Of particular interest are the male genitallic traits which may be of significance with respect to speciation in this group (Otte 1981). A DNA derived phylogeny will afford an opportunity for examining the evolution of such characters.



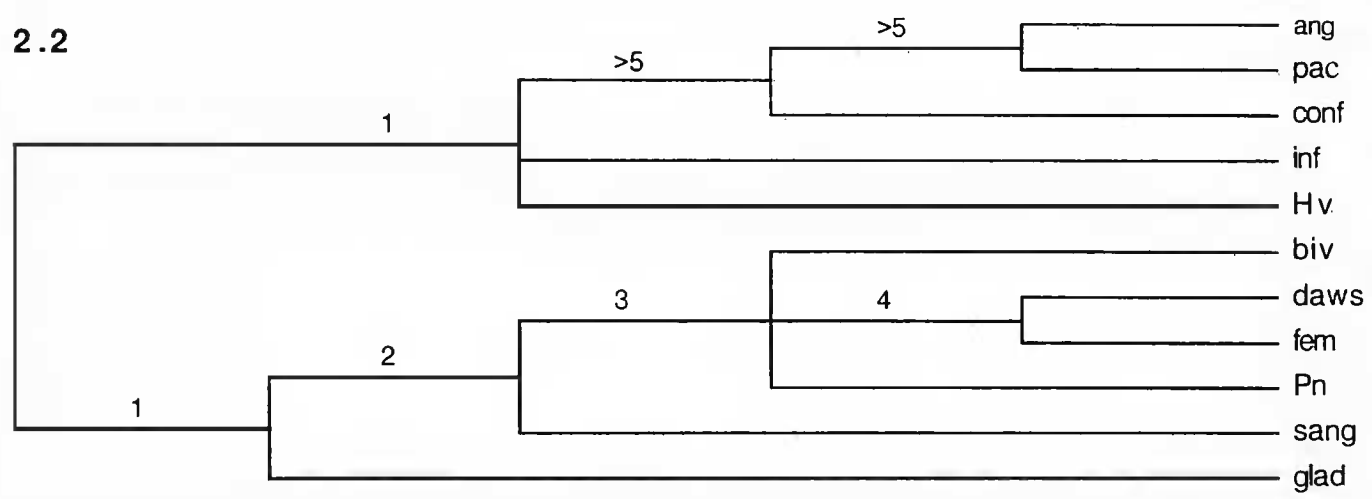


Figure 2.1. A single most parsimonious tree depicting relationships among 11 melanopline grasshopper species using all 22 traits. Tree length is 136 steps and consistency index is 0.54.

Figure 2.2. (Same data set with all characters treated as unordered) Majority rule consensus of 11 equally parsimonious trees, each of length 94 and consistency index 0.78. Numbers on branches in both figures refer to decay indices. Taxon abbreviations are defined in Table 2.

Acknowledgment

This work was supported by grants from the Faculties of Science and Graduate Studies and Research, University of Regina. Thanks are extended to N. W. Ashton for comments on the manuscript.

Literature Cited

Brooks, A. R. 1958. Acridoidea of Southern Alberta, Saskatchewan and Manitoba (Orthoptera). Can. Ent. Supp., 9: 1–92.

Buth, D. G. 1984. The application of electrophoretic data in systematic studies. Ann. Rev. Ecol. Syst., 15: 501–522.

Chapco, W. 1984. Inheritance of lactic acid dehydrogenase in *Melanoplus sanguinipes* (F.) (Orthoptera: Acrididae). Ann. Entomol. Soc. Am., 77: 11–13.

Chapco, W. 1989. Comparative variation at four enzyme loci in ten melanopline grasshoppers. Experientia, 45: 196–198.

Chapco, W., M. J. Bidochka & F. R. H. Hodson. 1987. Further genetic studies of *Melanoplus sanguinipes*. pp. 177–183. *In* Baccetti, B. (ed.). Evolutionary biology of orthopteroid insects. Horwood, Chichester, U.K.

Donoghue, M. J., R. G. Olmstead, J. F. Smith & J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on rbcL sequences. Ann. Missouri Bot. Gard., 79: 333–345.

Guenther, S. J. 1994. Evolutionary relationships amongst melanopline grasshoppers. M.Sc. Thesis, University of Regina, Regina, Canada.

Handford, R. H. 1946. The identification of nymphs of the genus *Melanoplus* of Manitoba and adjacent areas. Sci. Agric., 16: 147–180.

Harrison, R. G. 1986. Pattern and process in a narrow hybrid zone. Heredity, 56: 337–349.

Helfer, J. R. 1987. How to know the grasshoppers, crickets, cockroaches and their allies. Dover Publications, Mineola, New York.

Maddison W. P. & D. R. Maddison. 1992. MacClade: analysis of phylogeny and character evolution (Version 3.0). Sinauer Associates, Sunderland, Massachusetts.

Maurer, B. A., J. H. Brown & R. D. Rusler. 1992. The micro and macro in body size evolution. Evolution, 46: 939–953.

Onsager, J. A. & G. B. Mulkern. 1963. Identification of eggs and egg-pods of North Dakota grasshoppers (Orthoptera: Acrididae). Tech. Bull. N. Dakota St. Univ. Agric. Exp. Stn., 446: 1–48.

Otte, D. 1981. The North American Grasshoppers. Volume 1. Harvard University Press, Cambridge, Massachusetts.

Quicke, D. L. J. 1993. Principles and Techniques of Contemporary Taxonomy. Chapman & Hall, London, UK.

Simon, C. 1983. A new coding procedure for morphometric data with an example from periodical cicada wing veins. pp. 378–382. *In* Felsenstein, J. (ed.). Numerical taxonomy, NATO ASI Series, Vol. G1, Springer-Verlag, Berlin.

Swofford, D. L. 1993. PAUP: Phylogenetic Analysis Using Parsimony (Version 3.1.1). Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Vickery, V. R., & D. K. McE. Kevan. 1986. The grasshoppers, crickets and related insects of Canada and adjacent regions. Ulonata: Dermaptera, Cheleutoptera, Notoptera, Dictuoptera, Grylloptera and Orthoptera. The Insects and Arachnids of Canada, Part 14. Res. Branch, Agric. Canada Publ. No. 1777.

Received 19 Jun 1996; Accepted 12 Dec 1997.